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COMPUTATIONAL CHARACTERIZATION OF POROUS AND MECHANICAL PROPERTIES OF 3D SCAFFOLDS FOR POTENTIAL TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

In developing 3D tissue engineering (TE) scaffolds via rapid prototyping system, the design parameters such as filament gap, filament diameter and lay down angle which play significant roles in controlling porous and mechanical characteristics can be modulated. This study focuses on developing a computational model to simulate porous and mechanical characteristics of 3D tissue engineering scaffolds. The simulation is performed by manipulating the design inputs and analyzing the influences of change of the parameters on porous and mechanical characteristics of the scaffolds. With a constant filament gap, the increase of filament diameter decreases porosity and thus increases the mechanical properties of the scaffolds. However, with a constant filament diameter, the increase of filament gap increases the porosity and consequently, decreases the scaffolds' mechanical properties. Increasing lay down angle also increases the porosity that also influences the mechanical properties of the scaffolds. The actual mechanical properties of scaffolds are always obtained through physical experiments. The computational model provides predictive insight to the mechanical properties of the scaffold with specific design to be fabricated. Also, by superimposing the graphs of similar porous and mechanical characteristics from the computational model, the desired porous and/or mechanical properties can be obtained to design scaffolds as required. Utilizing the information obtained from the predictive model, the rapid

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prototyping technique could be employed to develop scaffolds with customized architecture and properties for any particular application.

KEYWORDS: Computational Characterization, Design Parameters, Porous and Mechanical Properties, Tissue Engineering, Scaffold, Rapid Prototyping

1. INTRODUCTION

Tissue Engineering (TE) scaffold serves as a template to facilitate cell adhesion and proliferation providing temporary mechanical support to newly grown tissue, while maintaining a 3D-structure. Tissue engineering aims to produce patient-specific biological substitutes to overcome the limitations of conventional clinical therapies. By transplanting cells onto scaffolds, experimental manipulation at three levels to achieve optimal construct is required. These levels are at the cells, the polymeric scaffolds and the construction method (Marler et al., 1998; Leong et al., 2003,).

With the technological advancements, there is a paradigm shift of interest from conventional scaffold fabrication techniques to RP techniques. It is difficult to produce scaffolds with great accuracy and reproducibility using conventional fabrication techniques. Scaffolds produced by conventional techniques such as fiber bonding, phase separation and solvent casting, tend to possess random and disordered micro-architectures and lack of mechanical strength (Bergman and West, 2008). Besides, the conventional techniques are associated with shape limitations as they have to be fabricated using molds and also being inconsistent and inflexible, rendering reproducibility of similar scaffolds extremely difficult (Bergman and West, 2008). The architecture, mechanical and porous characteristics of scaffolds produced by conventional techniques are often very difficult to predict and model.

Rapid prototyping (RP) is an automated process that can develop 3D scaffolds by sequential delivery of energy and/or material to points on the plane (Chua et al, 2003; Hoque et al., 2012). There is no need for any tooling or skilled craft model makers. An initial computer-aided design (CAD) model is input into a computer system. The model is then virtually sliced into multiple layers to lay out the fabrication work path by an RP software and lastly the designed model is fabricated in an additive manner layer by layer from the bottom (Wang et al., 2010; Almeida and Bártolo, 2010; Hoque et al., 2008).

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Precise parameters can be set up for advanced RP techniques, while the accuracy can be monitored by a computer program. As such, the growth rate of cells that are seeded in vitro or in vivo, and degradation of the scaffolds can be predicted more accurately. This is because the parameters of the scaffolds produced by RP techniques can be precisely quantified compared to scaffolds produce by conventional techniques. RP system is highly beneficial as it provides control over selecting the optimal scaffold design for optimal cell growth, while maintaining structural integrity upon application.

There are number of RP techniques that are widely used for commercial and research purposes such as stereolithography (SLA), fused deposition modeling (FDM), laminated object manufacturing (LOM) and many others. However, the growth of science in the RP field has led to the development of more contemporary techniques. These techniques include multi-nozzle deposition manufacturing (MDM), desktop robot based rapid prototyping (DRBRP) system, robocasting, pressure-assisted microsyringe etc. which are innovative improvements from its predeceasing technologies (Almeida and Bártolo, 2010; Hoque et al., 2011a).

Predictive computational models are used to bridge gaps between the supply and demand of the scaffold design. In order to develop an optimal scaffold for a particular application, the scaffold has to possess appropriate mechanical and porous characteristics (Hoque et al., 2011b). Computational models provide quick means to obtain and cross reference the scaffold design output data with specific porous and mechanical requirements for a particular TE application.

In essence, with an appropriate incorporation of computational model and RP technology, tailored scaffolds could be developed. This would provide innovative solutions to real life problems allowing customization of treatment to patients suffering from diseased or damaged organs. As such, this study mainly focuses on the development of computational models to assist building scaffolds using extrusion-based rapid prototyping technique.

2. MATHEMATICAL MODELS FOR SCAFFOLD CHARACTERISTICS

2.1. POROUS AND MECHANICAL CHARACTERISTICS

2.1.1. POROSITY

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Scaffold porosity can be defined as empty space, which is argued to influence the mechanical strength and permeability of scaffolds. It is usually measured in a form of fraction or percentage. Armillotta and Pelzer (2007) define porosity as the ratio of scaffold voids to its total volume as stated below:

$$P = 1 - \frac{V_{solid}}{V_{cubic}} \qquad (1)$$

Where,

P	=	Porosity
V_{solid}	=	Volume of solid in scaffold
V_{cubic}	=	Gross volume of a scaffold cube

This mathematical formula (in terms of volume) is too generic to be considered in CAD data to hold proper control over the overall scaffold fabrication. It can be better illustrated in terms of unit cells within the scaffold. Depending on the scaffold volume, the number of cells within a scaffold is determined by the filament gap and filament diameter which when combined, controls the volume. Thus by decomposing the formula in terms of filament gap and filament diameter, a quadratic regression model (Montgomery, 1996) was derived empirically through experiments by (Ang et al., 2006) to further relate the scaffold's porosity to the filament diameter and filament gap.

$$P = 54.1 + 66.5F_{gap} - 58.9F_{diameter} - 20.2F_{gap}^2 + 23.3F_{diameter}^2 - 13.1F_{gap}F_{diameter} \qquad (2)$$

Where,

F_{gap}	=	Filament Gap
$F_{diameter}$	=	Filament Diameter

This model defines the filament gap as the centre to centre distance between adjacent filaments. Upon analysis using the pore size theory modelled by Naing et al. (2005) the filament gap creates voids that can essentially be modelled to the pore size for greater mathematical flexibility. The pore diameter is assumed to be equivalent to the largest circle that can be manifested within the geometry of a cubic or triangular pore. Combining both theories, Ang and co-researcher's (2006) model can possibly be more accurate by substituting the filament gap with the actual scaffolds' pore size. Therefore,

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the regression model replaces the filament gap with pore diameter, P_{gap} as shown in the modified regression model below:

$$P = 54.1 + 66.5P_{gap} - 58.9F_{diameter} - 20.2P_{gap}^2 + 23.3F_{diameter}^2 - 13.1P_{gap}F_{diameter} \quad (3)$$

Where,

P_{gap} = Pore Diameter

2.1.2. YOUNG'S MODULUS

The Young's modulus, E also known as modulus of elasticity or elastic modulus is a measure of rate of change of stress over strain (Hutchings, 1996) that can be stated as:

$$E = \frac{\text{tensile strength}}{\text{tensile strain}} = \frac{\sigma}{\varepsilon} \quad (4)$$

The Young's modulus for a scaffold however, can be modelled by assuming the scaffold to take a cubic or isotropic form (Hollister, 2005; Hollister and Lin, 2007).

For a cubic scaffold with a base material, E_{cubic}, upper can be modelled as:

$$E_{cubic,upper} = \frac{2E\rho(2-v)}{\rho(3v^2+v-2)-3v^2-3v+6} \quad (5)$$

On the other hand, for a general isotropic scaffold, Eisotropic, upper can be modelled as:

$$E_{isotropic,upper} = \frac{2E\rho(7-5v)}{\rho(3v^2+v-2)-3v^2-3v+6} \quad (6)$$

Where,

E_{cubic}, upper = Young's Modulus for Cubic Scaffold

$E_{isotropic}$, upper = Young's Modulus for Isotropic Scaffold

ρ = Volume fraction of Solid Material

v = Poisson's Ratio

The equations essentially take into consideration the material's bulk properties which would affect the mechanical characteristics of the scaffold. The mathematical model selected to better represent a wider variation of scaffold design is presented in Eq. (6).

2.1.3. YIELD STRENGTH

Yield strength is defined as the stress at which a material or structure begins to deform plastically. It is typically the stress at the yield point of which often occurs with a plastic

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strain of 0.2%. Ang et al. (2006) have carried out experiments to determine the relationship between the scaffold design and its mechanical properties. The analysis strongly suggests that the porosity directly influences the yield strength of the scaffold and is modelled as:

$$\sigma_c = -25.8 \ln P + 116.9 \quad (7)$$

Where,

σ_c = Yield Strength

Eq. (7) is used to model yield strength as it uses the modified porosity model shown in Eq. (3), which is now able to establish data for a wider range of scaffold designs. Eq. (7) also simplifies the correlation between design inputs and the yield strength of the scaffold that is only to be affected by porosity.

2.2. ASSUMPTIONS FOR THE COMPUTATIONAL MODEL

2.2.1. SCAFFOLD ASSUMPTIONS

Several assumptions are made while modeling the scaffold's properties based on its mechanical characteristics. The assumptions for the layout of the scaffold's design parameters are shown in Fig. 1a. As the mathematical models are formulated under ideal circumstances, the parameters should also match ideal conditions as closely as possible.

Firstly, the extruded filament diameter is assumed to have square cross-section. This is to maximize the contact area between the interlayer filaments. As can be seen from Fig. 1b, a filament diameter with a circular cross-section will have least contact area among the layers. This induces high stress at the contact points between the filaments. Hence, the filaments are modelled with a square cross section to maximize the contact area between the interlayer filaments. As such, ideally there will be no stress concentration effect in the system.

2.3. UNIT CELL CHARACTERIZATION

Fig. 1a also illustrates that the filaments are laid exactly over each other so that the junction behaves as a load bearing column under compression. As a result, the stress applied onto the scaffold can be supported by the "columns" and consequently be redistributed into the scaffold structure.

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2.3.1. 0°/90° LAY DOWN PATTERN

A typical scaffold with a 0°/90° lay down pattern can be seen in Fig. 2a. Unit cell models were generated using Pro/Engineer as illustrated in Fig. 2b-2c. The scaffold with a 0°/90° lay down pattern is modelled as an ideal cube as shown in Fig. 2b. On top of that, Fig. 2c represents the solid entities contained in a single unit cell volume, which is used to determine the solid volume in the scaffold.

2.3.2. 0°< Θ < 90° LAY DOWN PATTERN

A typical scaffold with a 0°< θ <90° lay down pattern can be seen in Fig. 3a. The triangular unit cell models were also generated (Fig. 3) using Pro/Engineer and the scaffold with triangular pore architecture is modelled to represent a triangular column based on previous assumptions. Fig. 3c represents the solid entities contained in a single unit cell of a scaffold with triangular pore architecture. This is used to determine the solid volume of the scaffold.

2.4. COMPUTER MODEL FLOW CHART

The computer model flow chart is presented in Fig. 4. Initially the lay down angle is input along with other design parameters of the scaffold (filament gap, filament diameter, scaffold dimensions, material properties). Depending on the lay down angle input, either the cubic or triangular equation path will be activated, providing the necessary details and predictive results for the selected scaffold design. Finally, it will be prompted to further continue running the simulation before going through the whole process again or ending the simulation process.

2.5. COMPUTATIONAL SIMULATION METHODOLOGY

To better identify the design constraints and trends based on manipulation of the filament gap, filament diameter and lay down angle, the material properties are assumed to be fixed. Hypothetical data of material properties are used to analyze the mechanical and porous characteristics of the scaffold in detail. The scaffold is modelled considering the width, length and height of 5 cm each, while the hypothetical Young's modulus and Poisson ratio of the material were assumed to be 2 GPa and 0.4, respectively.

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The filament gap is first kept constant, while slowly increasing the filament diameter from 0.1 – 0.5 mm with 0.1 mm increment. It produces the data (graph) that investigates the influence of filament diameter on the porous and mechanical characteristics. Likewise, the filament gap is increased with similar values, while keeping the filament diameter constant to investigate the influence of filament gap on the porous and mechanical characteristics. These graphs can then be superimposed to obtain a logical solution for designing a scaffold with required porous and mechanical characteristics.

3. COMPUTATIONAL RESULTS AND DISCUSSIONS

3.1. POROUS CHARACTERISTICS

The influences of user-defined design parameters namely, filament diameter, filament gap and lay down angle are studied to modulate the scaffold characteristics for various applications as required. It is observed that the variations of these parameters induce the scaffold's porous characteristics as demonstrated by Fig. 5 and 6. By keeping the filament gap constant and increasing the filament diameter, the porosity is found to decrease as shown in Fig. 5. Likewise, the increase of filament gap, while the filament diameter is kept constant results in increase of porosity as presented in Fig. 6. This is because of the fact that when the filaments are laid further from each other the bulk scaffold contains less deposited solid material, which makes more empty spaces in the scaffold resulting in higher porosity. On the other hand, when the filaments are laid closely there results in an increased deposition of solid material and thus accordingly, decreases the pore volume (Moroni et al., 2006; Sun et al., 2005; Hutmacher et al., 2004). The change of lay down angle also influences the scaffold's porous characteristic. For example, an increase of lay down angle from 30° to 60° increases the porosity by about 0.8-1% while the filament diameter varies and filament gap remains constant as demonstrated in Fig. 5.

However, the porosity increases by approximately 15% when the lay down angle increases from 30° to 90° with the same conditions of filament diameter and gap. Similarly, the increase of lay down angle from 30° to 60° increases the porosity by about 1.4-2.2% while the filament diameter remains constant and filament gap varies as demonstrated in Fig. 6. However, the porosity increases by approximately 20% when

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the lay down angle increases from 30° to 90° with the same conditions of filament diameter and gap. Interestingly, in Fig. 6, as the filament gap decreases towards zero the porosity curves tend to converge to a single point. This indicates that with zero filament gaps all the scaffolds will produce zero porosity irrespective of lay down angle. In Fig. 5 & 6, it is shown that the porosity changes significantly with the change of filament diameter and filament gap. Besides, different RP techniques have different levels of accuracy that might affect the scaffold characteristics as well. Therefore, there has to be stringent control over the filament diameter, filament gap, lay down angle and the manufacturing accuracy while designing a scaffold to obtain desired characteristics.

3.2. MECHANICAL CHARACTERISTICS

The influences of filament diameter, filament gap and lay down angle on the mechanical characteristics of the scaffolds are thoroughly investigated. The mechanical characteristics (Young's modulus and yield strength) of the scaffolds with varying filament diameter, filament gap and lay down angle are presented in Fig. 7 - 10. The scaffold porosity decreases with the increase of filament diameter while the filament gap remains constant. The decrease in porosity increases the scaffold's mechanical properties (Young's modulus & yield strength) as observed in Fig. 7 and 8. On the other hand, the scaffold porosity increases with the increase of filament gap while the filament diameter remains constant. The increase in porosity decreases the scaffold's mechanical properties (Young's modulus & yield strength) as observed in Fig. 9 and 10. The increase of lay down angle also increases the porosity of the scaffold and accordingly, decreases the mechanical properties as shown in Fig. 7 – 10. Therefore, with a constant filament diameter, the scaffold with smallest filament gap is found to be the stiffest and that with largest filament gap is found to be the weakest. This variation in mechanical properties due to change of filament gap can be attributed to the fact that the filament junctions mainly resist the deformation when the scaffold is compressed. Under compression, these junctions behave like columns. The scaffold with the smallest filament gap has maximum number of columns (i.e. junctions) in a given sample dimension that resist the deformation most and thus, the scaffold becomes stiffest. On the contrary, the scaffold with the largest filament gap has minimum number of columns in the same given sample dimension that resist the deformation least, rendering the scaffold weakest. Similar findings are reported in the experimental study

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performed by Moroni et al. (2006), which states that the dynamic stiffness and equilibrium modulus increase with decrease of fiber spacing. The observed trends are directly related to the scaffold porosity. The increase of filament diameter results in increase of solid material in the scaffold structure and accordingly, decreases the porosity and hence increases the mechanical strength (Young's modulus and yield strength) as shown in Fig. 7 and 8.

Results indicate that a customized scaffold can be designed through appropriate combination of the user-defined parameters such as filament diameter, filament gap and lay down angle for tailored tissue engineering application.

4. DEVELOPMENT OF SCAFFOLD

A systematic process can be generated to develop scaffolds by using similar characteristic graphs that can be superimposed. It can be used as a tool, like the steam table where the mechanical or porous outputs can be cross referenced and checked with design inputs and requirements to verify whether it meets the design specifications. However, the limitation of the mathematical model presented in this study is that it is only designed for the scaffolds with filament junctions that act as vertical columns perpendicular to the scaffold layers. As such, it is rigid and is only able to provide results for the fundamental designs of a scaffold. A more integrated and comprehensive algorithm has to be developed in order to obtain results from scaffolds with the basic cubic or prism unit cell designs. This computational model is designed for scaffolds with an ideal and distinct shape. Practically, the fabrication techniques are not always able to produce scaffolds with ideal resolutions due to its accuracy limitations. Thus, there might be disparities between the predicted and actual results of the scaffolds' mechanical and porous characteristics. Future study is aimed to examine the disparity between predicted and actual results of the scaffolds' mechanical and porous characteristics utilizing some available RP technologies. Also, a more comprehensive algorithm could be designed to further improve the range and dimensions of scaffold characteristic predictions.

With more detailed study (theoretical and experimental) and compilation of data, an effective scaffold library could be developed that would have an extensive database on the relationship among the scaffold materials, structures and properties. This would be

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an effective tool for the tissue engineers to design scaffolds for tailored tissue engineering applications. A scaffold library will allow users to easily select a scaffold's fundamental geometry and architecture as a point of reference and further modify its parameters to satisfy the requirements for a final biomedical application (Chua et al., 2003).

5. CONCLUSIONS

Based on the computational analyses, the manipulation of design parameters (filament gap, filament diameter and lay down angle) has significant influence on the porous and mechanical characteristics of the scaffold. The manipulation of filament gap has a higher order of control over the porous and mechanical characteristics of the scaffold as compared to filament diameter and lay down angle. Incorporating the data obtained from this predictive model into a suitable rapid prototyping technique, a patient-specific scaffold could be developed with customized structure and properties to satisfy wider tissue engineering applications.

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FIGURE CAPTIONS

Figure 1. a) Cross sectional view of ideal scaffold layout; b) Filament cross section variation & c) Plan view of scaffold lay down pattern angle (Moroni et al., 2006)

Figure 2. a) Microscopic image of a scaffold with internal architecture of a 0°/90° lay down pattern (Chua et al., 2003), b) Unit cell interpretation 0°/90° unit cell model, c) solid entities contained in a single unit cell volume

Figure 3. a) Microscopic image of a scaffold with a triangular internal architecture (Sun et al., 2005), b) Triangular unit cell interpretation triangle unit cell model, c) solid entities contained in a single unit cell volume

Figure 4. Flow chart dictating the process flow of the computer model used

Figure 5. Influence of change in filament diameter on porosity while the filament gap remains constant at 0.2 mm.

Figure 6. Influence of change in filament gap on porosity while the filament diameter remains constant at 0.2 mm.

Figure 7. Influence of change in filament diameter on Young's modulus while the filament gap remains constant at 0.2 mm.

Figure 8. Influence of change in filament diameter on yield strength while the filament gap remains constant at 0.2 mm.

Figure 9. Influence of change in filament gap on Young's modulus while the filament diameter remains constant at 0.2 mm.

Figure 10. Influence of change in filament gap on yield strength while the filament diameter remains constant at 0.2 mm.

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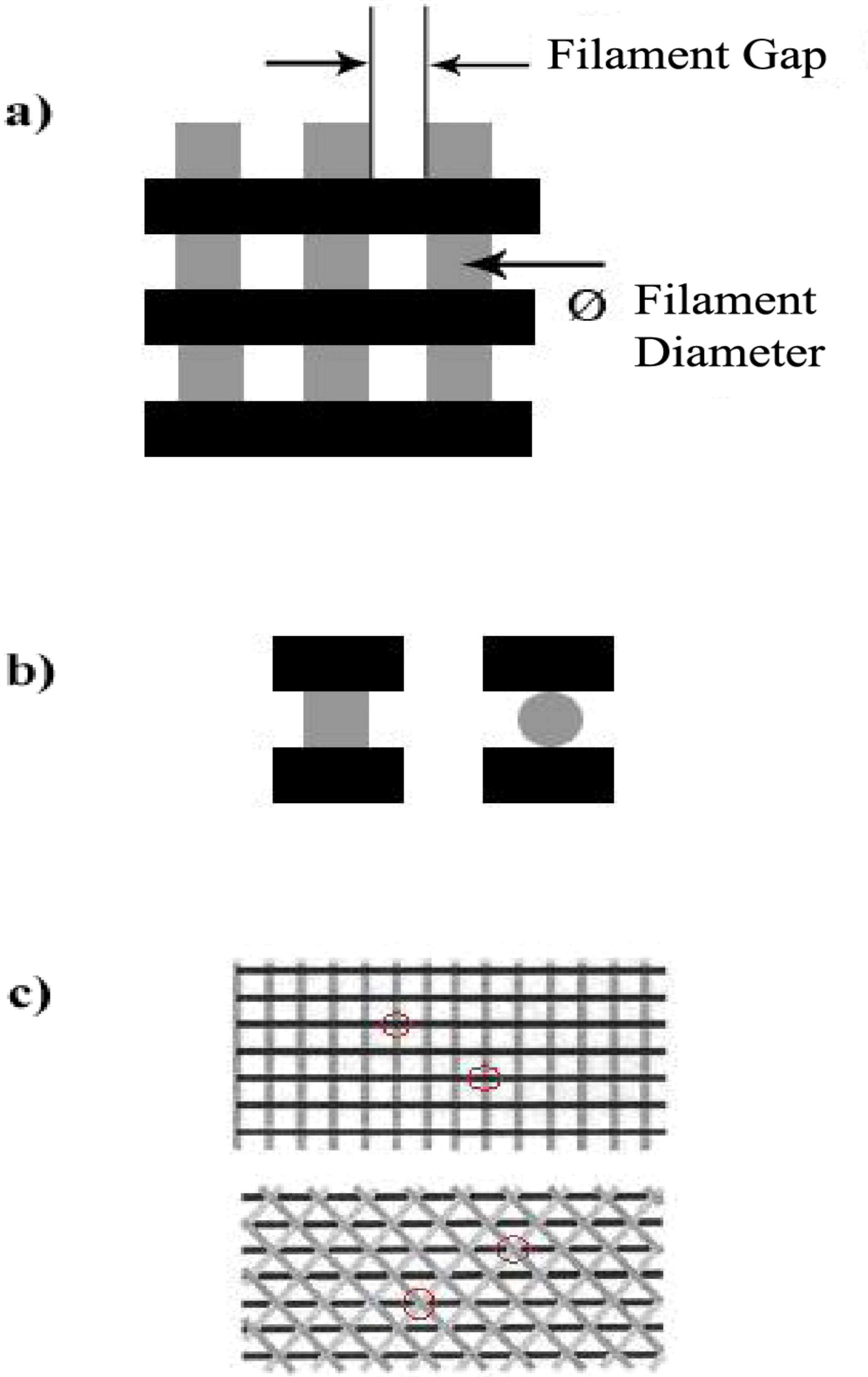

Figure 1

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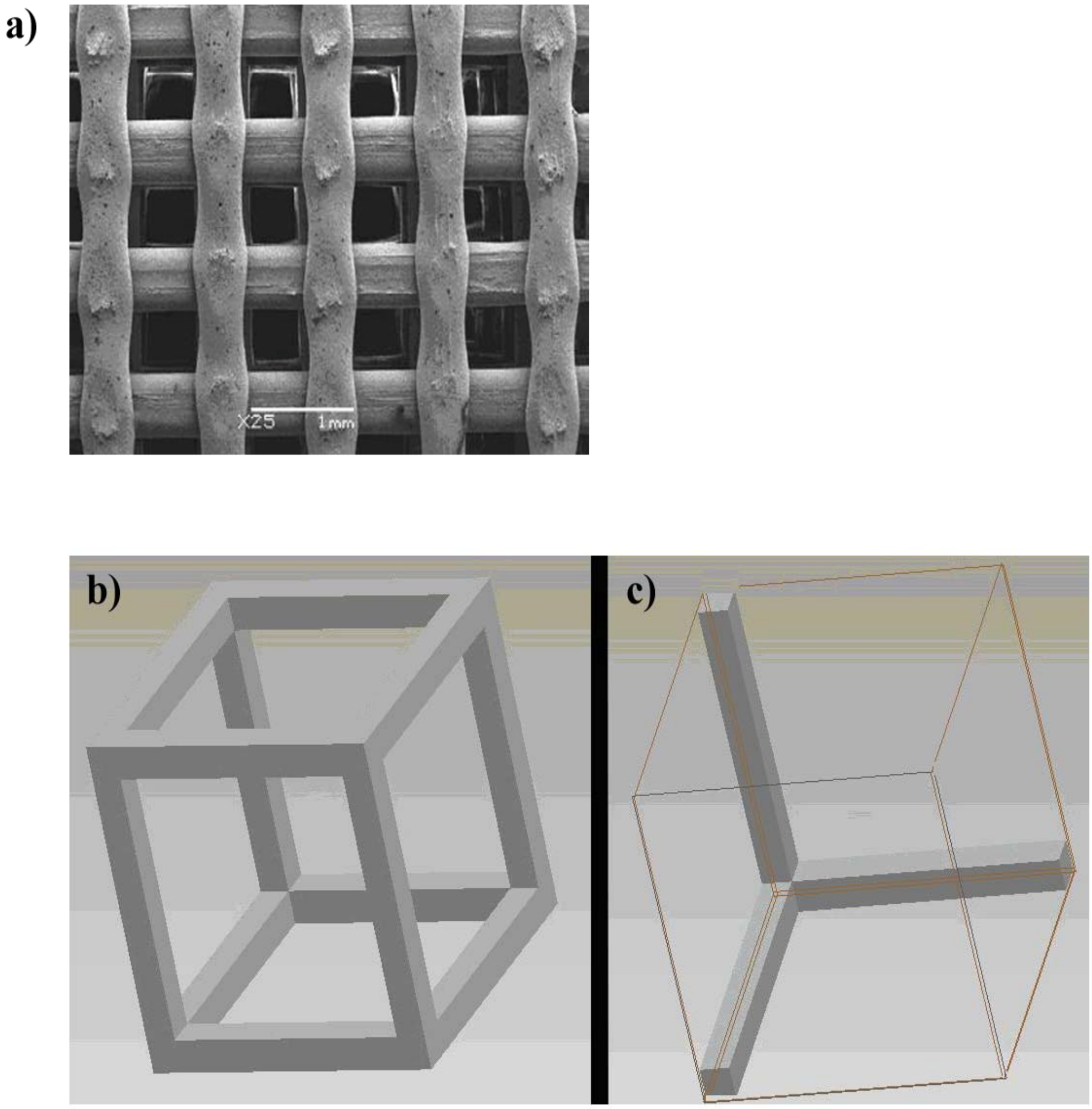

Figure 2

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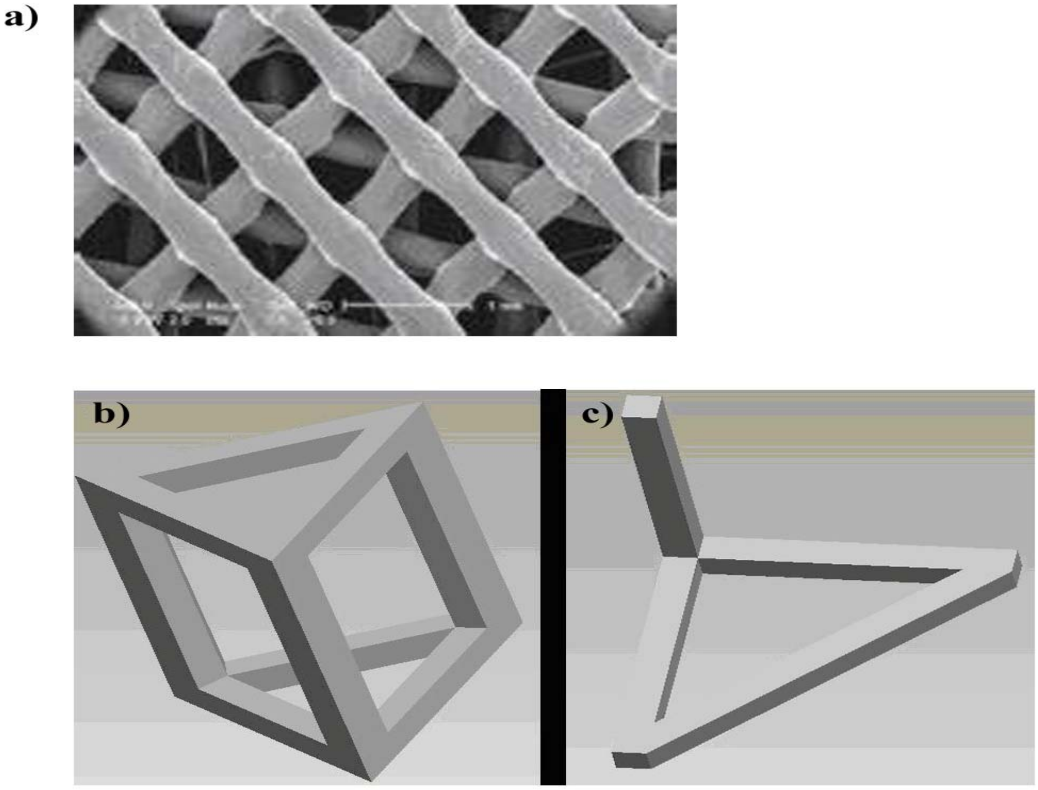

Figure 3

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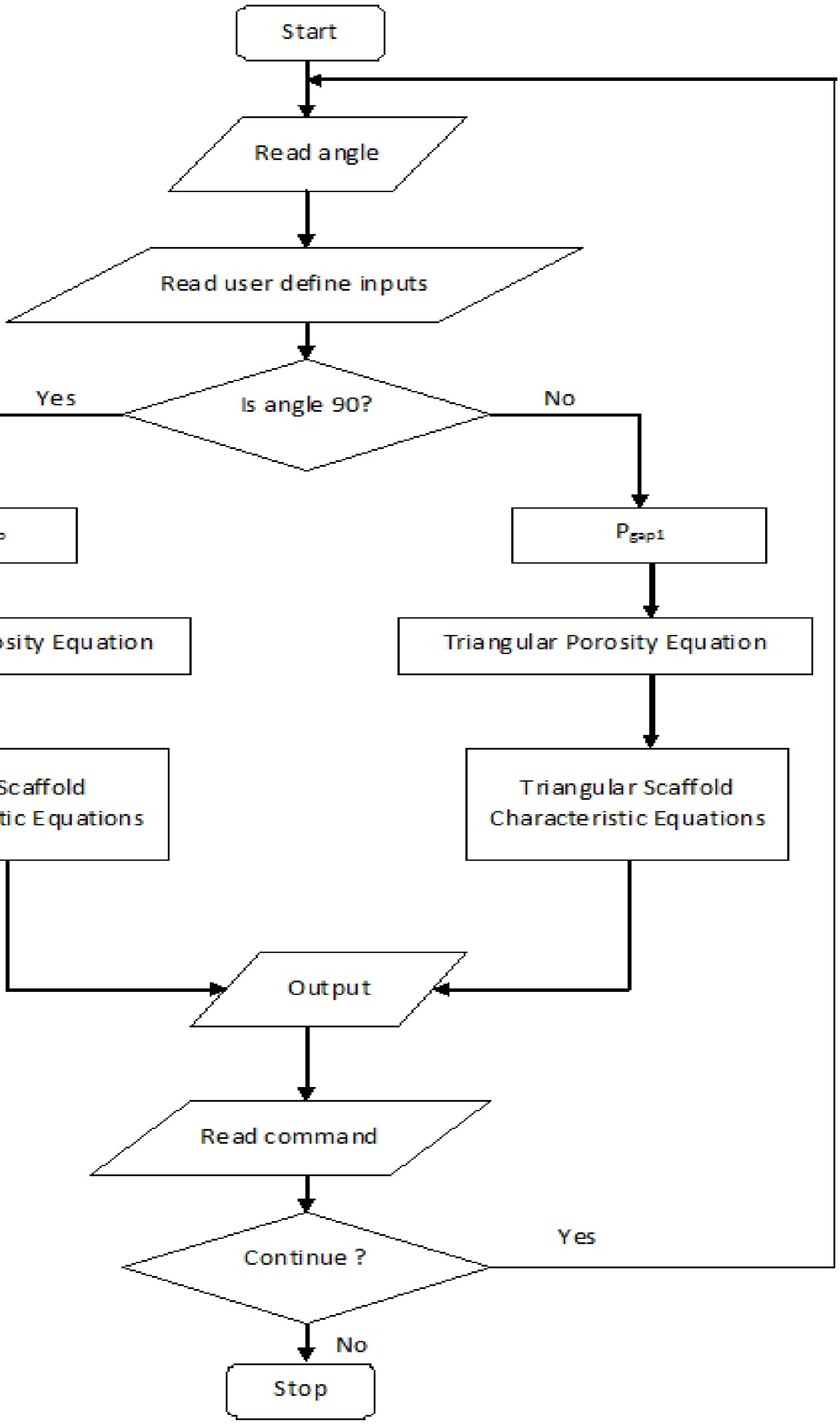

Figure 4

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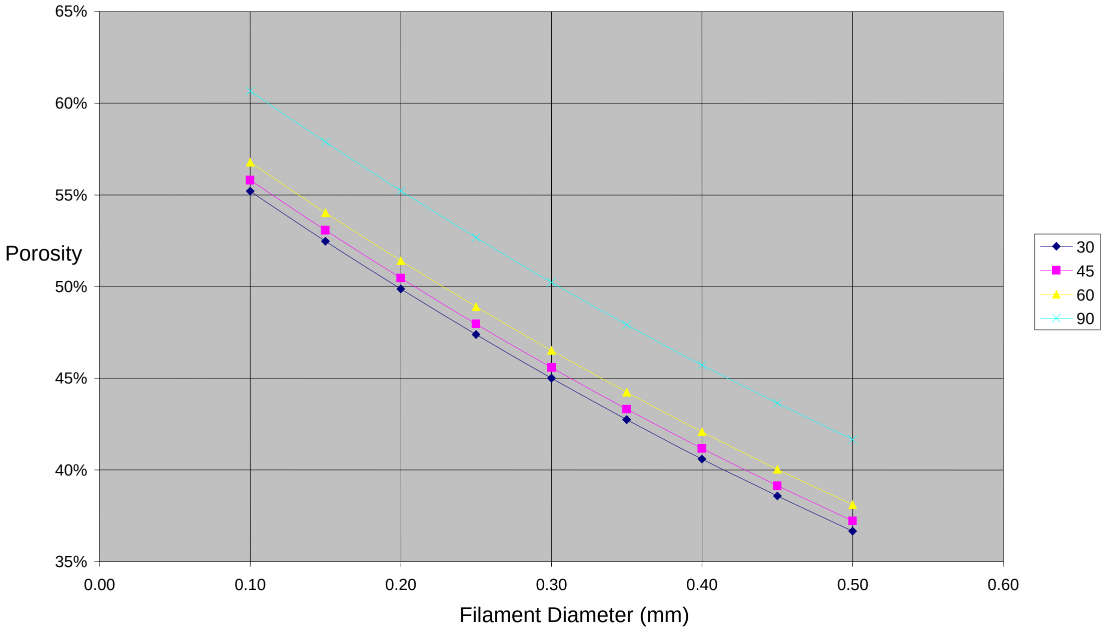

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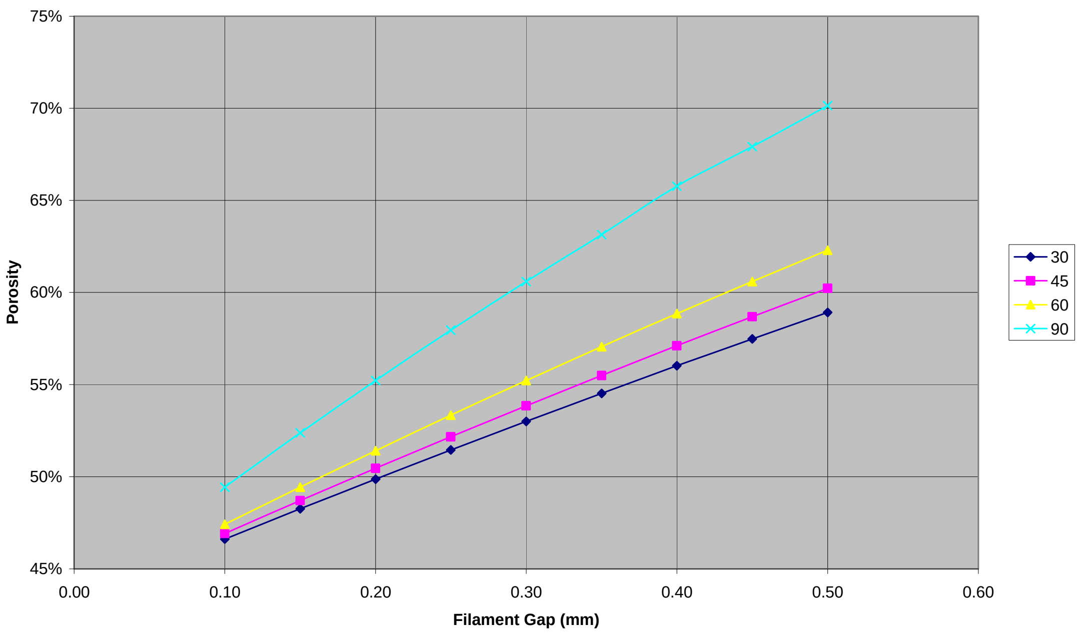

Figure 6

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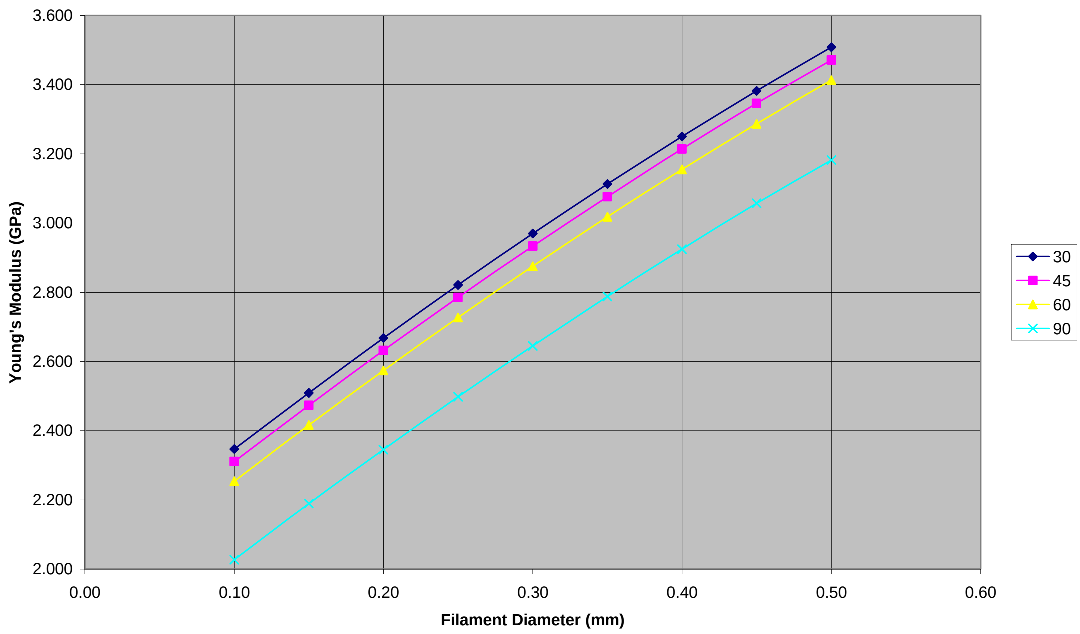

Figure 7

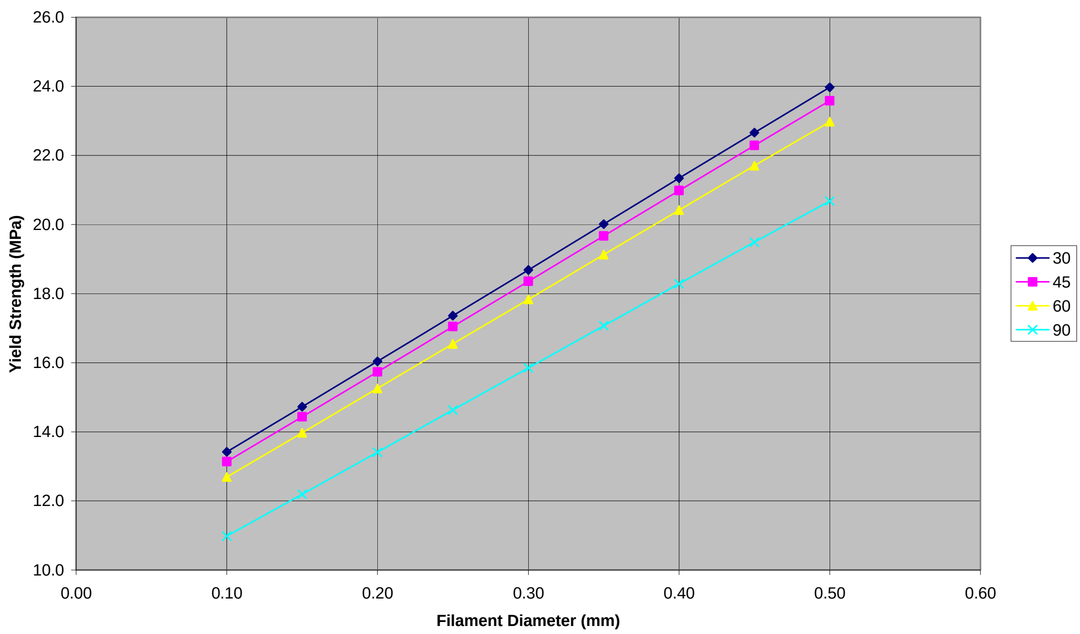

Figure 8

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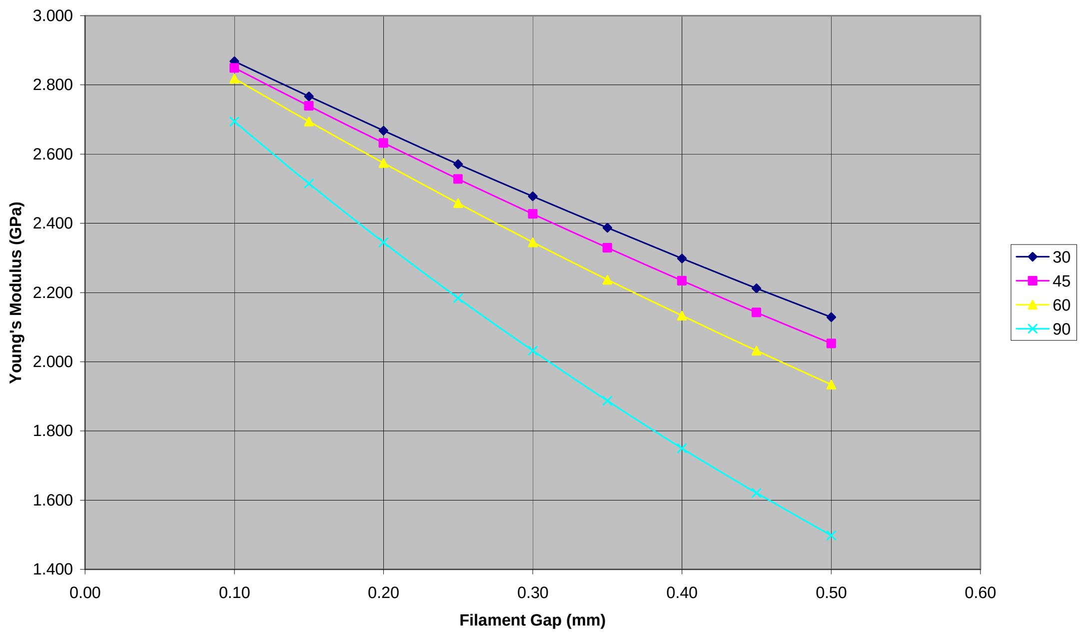

Figure 9

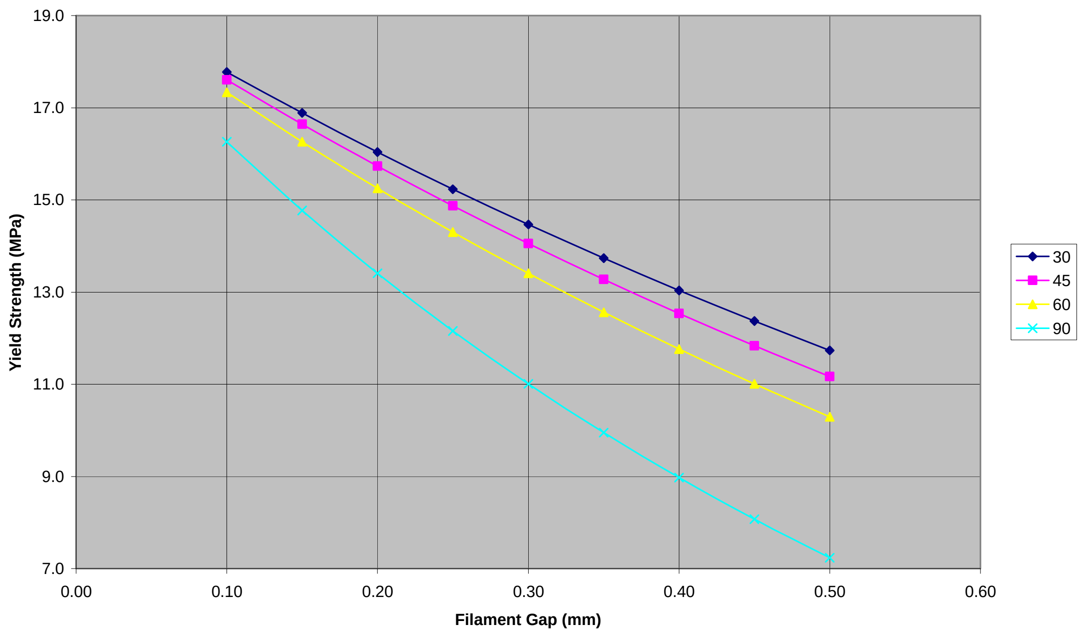

Figure 10